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With international search report.
With amended claims.

(54) Title: CLONING AND EXPRESSION OF A HUMAN α(1,3)FUCOSYLTRANSFERASE, FUCT-VI

(57) Abstract

A method for isolating a gene, comprising: (i) isolating a cell possessing a post-translational characteristic of interest, said post-translational characteristic being the presence of a membrane-bound oligosaccharide or polysaccharide of interest on the surface of said cell, the presence of a soluble oligosaccharide or polysaccharide of interest in an extract of said cell, or the presence of a particularly glycosyltransferase activity in an extract of said cell; (ii) creating a genetic library of either cDNA or genomic DNA from the genetic material of said isolated cell; (iii) transforming host cells with said genetic library; and (iv) screening said transformed host cells for a host cell containing said post-translational characteristic, thereby obtaining a cell containing said gene, is disclosed. The method can be used to obtain genes encoding glycosyltransferases.

sialylated type II oligosaccharides that can function as precursors to such molecules, via the action of the α(1,3)fucosyltransferase encoded by a transfected Lewis cDNA expression vector. COS-1 cells also maintain surface display of the type I precursors to the Lewis a (Galβ1→3[Fucα(1→4)]GlcNAc-) and sialyl Lewis a (NeuNAcα2→3Galβ1→3[Fucα(1→4)]GlcNAc-) moieties. The vector pCDNAI was used since this plasmid efficiently transcribes exogenous, subcloned sequences in mammalian hosts by virtues of the cytomegalovirus immediate early promoter sequences in the vector.

In initial biochemical analyses, extracts prepared from COS-1 cells transfected with plasmid pCDNAI-Fuc-TIV were tested for the presentce of vector-dependent fucosyltransferase activity, using several low molecular weight acceptor substrates. In a standard fucosyltransferase assay ("Experimental Procedures"), extracts prepared from pCDNA1-Fuc-TIV transfected cells, but not from control transfectants, contained a fucosyltransferase activity (296 pmol/mg-h) that utilized the type II disaccharide acceptor N-acetyllactosamine to yield a radiolabeled product with a chromatographic mobility ("Experimental Procedures") characteristic of authentic Galβ1→3[Fucα(1→4)]GlcNAc ($R_{2'\text{-fucosyl-N-acetyllactosamine}}$ = 0.85). However, under these assay conditions, two other neutral type II molecules (2'fucosyllactose, lactose) did not function as efficiently as N-acetyllactosamine as acceptor substrates for the fucosyltransferase in these extracts (17 and 10 pmol/mg-h, respectively, for 2'-fucosyllactose and lactose). Only a trace amount of transfer could be detected using the type I substrate lacto-N-biose I.

Likewise, the inventor did not detect fucose transfer to the sialylated acceptor NeuAcα(2→3)Galβ(1→4)GlcNAc (less than 1 pmol/mg-h), even in extracts that exhibited a relatively

large amount of activity toward N-acetyllactosamine (474 pmol/mg-h). By contrast, under these same conditions, extracts containing the Lewis blood group fucosyltransferase utilized both the sialylated acceptor (297 pmol/mg-h) and N-acetyllactosamine (526 pmol/mg-h), to form, respectively, the sialyl Lewis x tetrasaccharide and the Lewis x trisaccharide (see "Experimental Procedures"). Thus, the restricted acceptor preference exhibited by this enzyme *in vitro* contrasts remarkably with that exhibited by the Lewis α(1,3/1,4)fucosyltransferase, which can efficiently utilize each of the five acceptors tested. These results are summarized in Table 2.

COS-1 cells transfected with pCDNA1-Fuc-TIV were also analyzed by flow cytometry to detect *de novo*, vector-dependent surface expression of these oligosaccharide products, to allow an assessment of the enzyme's *in vivo* acceptor substrate requirements. The transfected COS-1 cells exhbited positive staining with a monoclonal antibody directed aginst the Lewis x moiety Galβ1→4[Fucα(1→3)]GlcNAc-) (Fig. 8), whereas cells transfected with the pCDNA1 vector without insert did not express this determinant. However, COS-1 cells transfected with pCDNA1-Fuc-TIV, or with its control plasmid, did not stain with antibodies specific for the sialyl Lewis x antigen (Fig. 8). Likewise, the transfected cells did not exhibit detectable surface expression of Lewis a or sialyl Lewis a molecules (Fig. 8).

Polylactosaminoglycans with terminal α(2→3)-linked sialic acid also exist that maintain a single internal α(1,3)-linked fucose on the N-acetylglucosamine residue of the pneultimate lactosamine repeat. This determinant (NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4[Fucα(1→3)]GlcNAc-) can be detected on the surfaces of myeloid cells by the monoclonal antibody VIM-2, **and may be constructed by the action of α(1,3)fucosyltransferase(s) on type II polylactosamine**

acceptors whose terminal galactose residues are substituted with α(2,3)sialic acid moieties. Neither COS-1 cells transfected with the Lewis α(1,3/1,4)fucosyltransferase, nor COS-1 cells tranfected with plasmid pCDNA1-Fuc-TIV display detectable amounts of this determinant.

Virtually identical results were obtained with COS-1 cells transfected with the plasmid pCDNAI-α(1,3)FTMlu ("Experimental Procedures"). This vector encompasses sequences corresponding to bp-1904 through the end of the open reading frame in Fig. 4. These results provide additional evidence for the hypothesis that the open reading frame displayed in Fig. 4 corresponds to the coding portion of this fucosyltransferase gene.

To further demonstrate that enzymatic activity is directly associated with this protein, the putative catalytic domain of the predicted polypeptide (amino acids 50 to 405 of SEQ ID NO: 8) was fused to a secreted form of the IgG binding domain of Staphylococcus aureus protein A in the mammalian expression vector pPROTA, to generate the vector pPROTA-α(Fuc-TIV)$_c$. Since this fusion protein would lack the putative transmembrane anchoring segment of the fucosyltransferase, the inventor expected it would be synthesized as a secreted molecule that could be affinity-purified on an IgG-containing matrix and subsequently tested for α(1,3)FT activity. COS-1 cells transfected with the control vectors pCDM7 or pPROTA generated no detectable cell-associated or released enzyme activity. However, conditioned media prepared from COS-1 cells transfected with pPROTA-α(Fuc-TIV)$_c$ contained significant quantities of α(1,3)FT activity when assayed with N-acetyllactosmine. Virtually 100% of the released activity generated by pPROTA-α(Fuc-TIV)$_c$ is specifically retained by the IgG-Sepharose matrix. These results indicate that the protein encoded by this cloned DNA segment encodes a fucosyltransferase, and demonstrate that information

Claims

1. An isolated DNA fragment comprising a sequence which encodes the amino acid sequence of SEQ ID NO:14.

2. The DNA fragment of Claim 1, which comprises the sequence from base 255 to base 1208 of SEQ ID NO:13.

3. The DNA fragment of Claim 1, which comprises SEQ ID NO:13.

4. A vector, comprising a DNA sequence which encodes the amino acid sequence of SEQ ID NO:14.

5. The vector of Claim 4, wherein said DNA sequence comprises from base 255 to base 1208 of SEQ ID NO:13.

6. The vector of Claim 4, wherein said DNA sequence comprises SEQ ID NO:13.

7. A protein having the sequence of SEQ ID NO:14.

8. A polypeptide, comprising an amino acid sequence corresponding to positions 43 to 359 of SEQ ID NO:14.

AMENDED CLAIMS

[received by the International Bureau on 21 December 1993 (21.12.93) ; new claims 9-13 added ; other claims unchanged (2 pages)]

1. An isolated DNA fragment comprising a sequence which encodes the amino acid sequence of SEQ ID NO:14.

2. The DNA fragment of Claim 1, which comprises the sequence from base 255 to base 1208 of SEQ ID NO:13.

3. The DNA fragment of Claim 1, which comprises SEQ ID NO:13.

4. A vector, comprising a DNA sequence which encodes the amino acid sequence of SEQ ID NO:14.

5. The vector of Claim 4, wherein said DNA sequence comprises from base 255 to base 1208 of SEQ ID NO:13.

6. The vector of Claim 4, wherein said DNA sequence comprises SEQ ID NO:13.

7. A protein having the sequence of SEQ ID NO:14.

8. A polypeptide, comprising an amino acid sequence corresponding to positions 43 to 359 of SEQ ID NO:14.

9. The DNA fragment of Claim 1, which consists essentially of a sequence which encodes the amino acid sequence of SEQ ID NO:14.

10. The DNA fragment of Claim 2, which consists essentially of the sequence from base 255 to base 1208 of SEQ ID NO:13.11. The DNA fragment of Claim 3, which consists essentially of SEQ ID NO:13.

12. The vector of Claim 5, wherein said DNA sequence consists essentially of from base 255 to base 1208 of SEQ ID NO:13.

13. The vector of Claim 6, wherein said DNA sequence consists essentially of SEQ ID NO:13.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06703

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 15/54, 15/85, 9/10
US CL :435/193, 320.1, 69.1; 536/23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/193, 320.1, 69.1; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, BIOSIS, MEDLINE, EMBASE, LIFESCI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 267, No. 34, issued 05 December 1992, B.W. Weston et al., "Molecular Cloning of a Fourth Member of a Human α(1,3)Fucosyltransferase Gene Family, pages 24575-24584, see entire document.	1-8
P,X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, Volume 187, No.1, issued 31 August 1992, K.L. Koszdin et al., "The Cloning and Expression of a Human α-1,3 Fucosyltransferase Capable of Forming the E-Selectin Ligand", pages 152-157, see entire document.	1-8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer ROBERT WAX Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, Volume 190, No.1, issued 15 January 1993, S. Nishihara et al., "Human α-1,3 Fucosyltransferase (FucT-VI) Gene is Located at Only 13 Kb 3' to the Lewis Type Fucosyltransferase (FucT-III) Gene on Chromosome 19", pages 42-46, see entire document.	1-8
X	JOURNAL OF CELLULAR BIOCHEMISTRY SUPPLEMENT, Volume 19, Part D, issued March 1992, B.W. Weston et al. "Defining a Glycosyltransferase Gene Family Cloning and Expression of a Gene Encoding a GDP-Fucose N-Acetylglucosamide 3-Alpha-L-Fucosyltransferase Homologous to But Distinct From Known Human α-1,3 fucosyltransferases. page 150, see entire document.	1-8
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 267, No. 6, issued 25 February 1992, B.W. Weston et al. "Isolation of a Novel Human α(1.3)Fucosyltransferase Gene and Molecular Comparison to the Human Lewis Blood Group α(1,3/1,4)Fucosyltransferase Gene" pages 4152-4160, see entire document.	1-8